Section of Comparative Medicine

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SYMPOSIUM ON RECENT DEVELOPMENTS IN IMMUNIZATION AGAINST BACTERIAL DISEASE

The Basis of Immunity to Anthrax

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IN this précis, various preparations which immunize animals against anthrax will be described, and their relationship to the factors which appear to determine the virulence of *Bacillus anthracis*, will be discussed.

NON-TOXIC IMMUNOGENIC PREPARATIONS FROM *B. anthracis*

Pasteur first vaccinated against anthrax using living attenuated cultures, and to-day live uncapsulated strains of *B. anthracis* (e.g. the "Sterne" strain) are still used for vaccinating bovines. The immunogenic products of the growth of *B. anthracis, in vivo*, were first demonstrated by Bail and Weil (1911) in the œdema fluid from infected animals. Watson *et al.* (1947) showed the immunogenic activity of such fluid was associated with the β and γ globulins and Smith and Gallop (1956) isolated from it an immunogenic, high molecular weight lipoprotein containing carbohydrate residues.

Attempts to produce a dead immunogenic preparation *in vitro* did not succeed until some thirty to forty years after the work of Bail and Weil. Gladstone (1946) finally produced an immunogenic culture filtrate in a serum medium. Later, an immunizing antigen was obtained in a synthetic medium, first by Wright *et al.* (1954), and then in greater yield by Belton and Strange (1954); it appeared to be a protein (Strange and Belton, 1954).

The preparations described above would actively protect guinea-pigs, rabbits and monkeys against anthrax but only appeared to have low-grade effect in mice; preliminary information from a trial being conducted at present on personnel from the goat hair industries of U.S.A. indicates that the immunizing antigen produced *in vitro* in a synthetic medium is effective in protecting man against anthrax (Brachman and Wright, 1958). In contrast to the immunogenic anthrax toxin described below, these various preparations neither produced œdema when injected intradermally nor were lethal when injected intravenously.

THE VIRULENCE FACTORS OF *B. anthracis*

The virulence of *B. anthracis* is determined by the presence of at least two factors—an extracellular toxin and capsular polyglutamic acid (for a complete series of references *see* Smith and Keppie, 1955; and Smith, 1958). The anthrax toxin was first recognized in the blood of guinea-pigs dying of anthrax but later was produced *in vitro;* it is lethal when injected intravenously and produces an extensive area of œdema when injected intradermally. The toxin enhances the virulence of an attenuated strain of *B. anthracis*, inhibits the phagocytosis of *B. anthracis* by guinea-pig polymorphs, and inhibits the action of an anthracidal substance present in some normal sera and in extracts of leucocytes; it therefore acts as an aggressin in the early stages of anthrax. The toxin is a powerful immunizing antigen.

Capsular polyglutamic acid has been isolated from organisms grown *in vivo*. Like the toxin, it is an aggressin but it is non-lethal and does not evoke œdema. Furthermore, neither isolated polyglutamic acid nor that present in dead capsulated organisms is immunogenic.

THE RELATIONSHIP OF NON-TOXIC IMMUNOGENIC PREPARATIONS TO THE TOXIN

Since the immunogenic toxin is produced by *B. anthracis, in vivo*, it appears to be the prime basis for immunity to anthrax. There is, however, a very strong connexion between this toxin and the two important non-toxic immunogenic preparations just described—the lipoprotein isolated from œdema fluid and the "*in vitro* antigen", i.e. the purified preparation (Strange and Belton, 1954) from culture *in vitro*.

Table I shows that there is a strong serological connexion between these two preparations and the anthrax toxin, because the latter is neutralized by sera prepared against the former. To follow the connexion farther, an important property of the anthrax toxin must be described. The toxin consists of two components; one (factor I) is deposited quickly during ultracentrifugation leaving the second (factor II) in the supernatant (Smith *et al.*, 1956). The

TABLE I.—SPECIFIC NEUTRALIZATION OF THE TOXIN OF *B. Anthracis*

Toxin injected after mixing with ⅓ vol. of:	Œdema in guinea-pig (0·2 ml. i/d)	Lethality in mice i/v 1 ml.	0·5 ml.
Saline	+++	25/25	23/25
Normal horse serum	+++	10/10	11/15
Horse antiserum (to spore vaccine) ..	Nil	0/15	0/15
Horse antiserum (to "*in vitro* antigen")	Nil	0/10	0/15
Guinea-pig antiserum (to lipoprotein) ..	Nil	0/5	—

two components act synergically in tests for œdema production and lethality (*see* Table II). Stanley and Smith (1958) and Sargeant and Smith (1958) have purified factors I and II using the cellulose ion exchange columns of Peterson and Sober (1956). The most purified sample of factor I is a lipoprotein containing carbohydrate residues, and purified factor II is largely protein. Both factors very easily lose their potential toxicity even at 0° C. but retain their antigenic character.

There is little doubt that the non-toxic lipoprotein formerly isolated from œdema fluid was factor I of the anthrax toxin which had lost its potential toxicity in the separation procedure; indeed, experiments on active factor I showed that such procedures did destroy its potential toxicity.

The "*in vitro* antigen" appears to contain components connected with both factors of the anthrax toxin. This is indicated by two lines of evidence. Firstly, the "*in vitro* antigen" (*a*) will replace factor II in the synergic mixtures with factor I for œdema production or mouse lethality tests and (*b*) will replace to some extent factor I in the synergic mixtures with factor II for mouse lethality tests (*see* Table II). Secondly, the "*in vitro* antigen" contains two com-

TABLE II.—SYNERGIC MIXTURES OF FACTOR I WITH FACTOR II OF THE ANTHRAX TOXIN AND WITH THE "*in vitro* ANTIGEN"

Material	Œdema production i/d, guinea-pig 0·2	0·1	0·05 ml.	Mouse lethality i/v 2	1	0·5	0·25 ml.
Factor I* alone	+++	++	+	—	0/5	0/5	0/5
Factor II* alone	+	Trace	Nil	6/25	1/25	0/25	—
"*In vitro* antigen"* alone ..	Nil	Nil	Nil	1/10	0/10	0/10	—
Mixtures							
Factor I .. .. .. + Factor II	0·05 ml. + 0·1 ml.		+++		0·25 ml. + 0·5 ml.		5/5
Factor I .. .. .. + "*In vitro* antigen"	0·05 ml. + 0·1 ml.		+++		0·25 ml. + 0·5 ml.		5/5
Factor II .. .. .. + "*In vitro* antigen"	0·1 ml. + 0·1 ml.		+	0·5 + 0·5	1·0 ml. + 1·0 ml.		2/5, 8/10

*13 ml. of original toxin produced 1·5 ml. factor I and 7 ml. factor II. The "*in vitro* antigen" of our colleagues Strange and Belton (1954) was made up at 0·1% w/v. All œdema production and lethality were specifically neutralizable by anthrax antiserum.

ponents which form common serological precipitation lines with factors I and II of the anthrax toxin in the standard procedure on Ouchterlony plates using anthrax antiserum prepared against the "*in vitro* antigen" (*see* Fig. 1) or against live spores. The relevant components

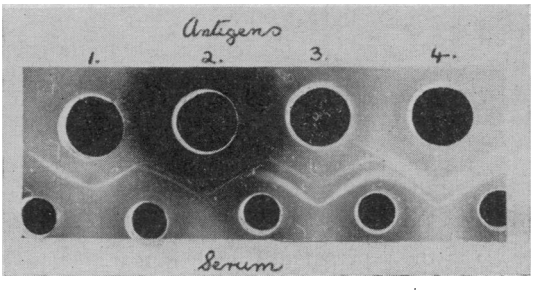

FIG. 1.—Serological precipitation lines on Ouchterlony plates. Antigen 1.—Purified factor I of anthrax toxin 0·3 mg. Antigen 2.—Crude anthrax toxin 8 mg. Antigen 3.—Purified factor II of anthrax toxin 2·6 mg. (this still contains approx. 2% of factor I). Antigen 4.—The "*in vitro* antigen" 0·3 mg. Serum—anthrax antiserum prepared against the "*in vitro* antigen" (a similar picture is obtained using antiserum prepared against live spores). With different batches of toxin and different sera the line corresponding to factor I sometimes appears as a double line.

in the "*in vitro* antigen" are not exactly factors I and II of the anthrax toxin. Thus, apart from the obvious fact that the "*in vitro* antigen" is non-toxic, this antigen when it acts as factor II will only increase the œdema production of an amount of factor I which has a small inherent effect; it will not, as does authentic factor II, form an œdema-producing mixture with a smaller and separately unreactive amount of factor I. Furthermore, the "*in vitro* antigen" has no synergic effect on factor II as regards œdema production (*see* Table II).

SUMMARY

Of the two main virulence factors of *B. anthracis*—namely the toxin and capsular polyglutamic acid—the toxin is immunogenic and forms the prime basis for immunity to anthrax. The toxin consists of two factors which act synergically, and lose their toxicity very easily while retaining their antigenicity.

A non-toxic immunogenic vaccine prepared *in vitro*—an excellent practical vaccine—contains components which are closely connected with both factors of the anthrax toxin.

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Strain 19 and Other Brucella Vaccines

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INTRODUCTION

Prior to the introduction of S.19 vaccine to this country in 1941, a broth culture *Br. abortus* vaccine had been issued from Weybridge for many years. Under certain circumstances this vaccine was probably fairly efficient but its antigenic properties were unknown and it was rightly replaced by the S.19 vaccine.

Vaccination with S.19, an attenuated smooth strain (originally isolated by Cotton and Buck in 1923), had been started some years earlier in the United States and, by 1939, it had become firmly established there for the control of bovine brucellosis. The production of the liquid vaccine was begun at Weybridge with the assistance and advice of the United States Bureau of Animal Industry and output rapidly increased until an annual production of three-quarter million 5 ml. doses was attained. In 1944, the Ministry of Agriculture introduced the Calfhood Vaccination Scheme, whereby heifer calves from the age of 6 months to breeding age could be vaccinated at a small charge to the farmer by his own veterinary surgeon or by the staff of the Ministry. The scheme proved to be very successful and to-day over half of the vaccine issued is used for calfhood vaccination.

METHODS OF VACCINE PREPARATION

In the preparation of the vaccine, care is taken to ensure that the smooth phase of the organism is always employed; the antigenicity of the vaccine is greatly reduced if dissociation is allowed to occur. For this reason the strain has always been cultivated on potato agar, although new techniques are now being evolved for production in a liquid medium; Roux flask culture is always laborious and the contamination risks are considerable. In 1956 the vaccine department at Weybridge moved into a new building equipped with the latest methods of ventilation for the production of a sterile atmosphere; the general improvements resulting from this have increased the yield by almost 20%.

Liquid culture techniques have been used by van Drimmelen (1956) at Onderstepoort for the production of S.19 vaccine. He has employed mechanical shaking of liquid cultures in large glass containers and, more recently (1957), the vortex system where 80 litres of liquid are revolved at 1,000 r.p.m. for seventy hours and a 2,000 to 6,000-fold increase in growth